

Estimated biomass of Ciliata (Protozoa) communities in alkaline soils of the Hortobágy National Park, Hungary

By

A. SZABÓ*

Abstract. Studies have been carried out in different subtypes of solonetz soils, forming a mosaic pattern. In the soils of the HNP the estimated biomass values are higher in the upper layers (0-5 cm) than in the lower ones (5-10-20 cm). Extremely high values (11-16 kg/ha) were experienced in the sampling sites with humous-rich upper soil-horizon. A 30-40% volume decrease has been recorded in the deeper soil layers. The estimated biomass of Ciliata in the sampling sites with eroded A-horizon is 1-3 kg/ha. Based on the biomass values, soils of the HNP can be ordered in the same way (I, VII, V, IV, III, VI sampling sites) as they have been in case of the species-individuals relations. Because of the mosaic pattern of solonetz soils, the estimated average Ciliata biomass, calculated on the basis of actual (not cultured) numbers of individuals, is estimated to be 6.6 kg/ha in the 0-5 cm layer. Although this value is lower than that calculated in other (e.g. chernozem, forest, pasture) soil types, our data demonstrate the importance of Ciliata populations even in the alkaline soils, and one has to take them into consideration while studying food chains and flow of nutrients.

Function and importance of organisms and their communities living in various biotopes can only be measured if one has information about their activities, role and position in the food-chain, and also if there are data on their biomass within the given ecosystem. Protozoa (Ciliata) usually appear in the biotopes in great numbers. It is not simply their presence, but also the biomass they represent, which gives them an important role in a biological community (e.g. in the edaphon).

Determination the biomass of Protozoa (Ciliata) is not a simple task. There have been already some measures to calculate their volume (Sebestyén, 1958) mainly by comparing them to geometric bodies. There are also some recent publications (Buitkamp, 1979; Foissner, 1981, 1995, 1998) giving some guidelines (sometimes concrete values) for the measurement of the biomass of Ciliata. There are several difficulties while calculating the biomass of Ciliata living in soils. One of the most important of these is that the size or volume of Protozoa living in alkaline soils is many times much smaller than that in water bodies, because of troglodytism. In addition, other characteristics of the biotope could influence the volume (cubic measure) of Ciliata. For this reason

*Dr. András Szabó, DE, AC, MTK Talajtani és Mikrobiológiai Tanszék (Department of Soil Sciences and Microbiology, Agricultural Centre, Debrecen University), 4032 Debrecen, Bőszörményi út 138, Hungary.

one can only very carefully apply exactly the same methods and values published by other authors. This was emphasised by Gellért (1957), Varga (1960), Buitkamp (1979) and Foissner (1981) as well.

In this study there are estimations on the biomass of Ciliata communities living in different subtypes of solonetz soils, forming a mosaic pattern in the area of the Hortobágy National Park (HNP), Hungary. It is hoped that this study can contribute to have a more objective view on the function of Protozoa (Ciliata) in a given community.

Materials and methods

Plant associations and soil types

Studies have been carried out in one of the strictly protected areas of the HNP in different subtypes of solonetz soils, forming a mosaic pattern and each can be characterised by a specific plant association. Sampling sites were directly neighbouring with alkaline depressions, and they have been assigned in the halophytic plant associations of a mosaic pattern, characterising the erosional succession series and the different subtypes of solonetz soils as well.

On the higher surfaces there are deep or medium layer meadow solonetz soils covered by *Achilleo-Festucetum pseudovinae* (I) plant association. The A-horizon of the soil is thick (15-30 cm) with a 3-4 % humus content. The A-horizon does not contain Na-salts and the pH is slightly acidic.

Going down to lower parts - parallel with the gradual thinning of the A-horizon - one can find the *Artemisio-Festucetum pseudovinae* (II) association on medium meadow solonetz soil. In the sloping direction a discontinuous type of this plant association develops (in Hungarian called "alkaline tussocks disposed by hand"). The high intensity drop-erosion leads to the development of the typical alkaline soil "shoulders" (small surface elevations), supported by the precipitation of amorphous silicate (SiO_2) in the B-horizon. Because of the complete erosion of the A-horizon, Na-salts get closer to the surface, which can be tolerated only the *Camphorosmetum annue* (IV) plant association.

The wetter crusted solonetz soil with precipitated silicate is covered by *Puccinellietum limosae* (V). Here the a-horizon of the soil is missing and the B-horizon is of a column structure. There is precipitated silicate (SiO_2) on the surface. Because of the erosion process, the upper humic soil is getting together on lower parts, where the *Pholiuro-Plantaginetum tenuiflorae* (VI) plant association develops. The soil here is brownish black with a 2.6% humus content and accumulation of Na-ions. The border of the wet depression, being the base level, is covered by the *Agrosti-Beckmannietum eruciformis* (VII) plant association. Here the A-horizon is considerably leached, the B-horizon is often with no structure. Na-salts are found only in deeper parts of the soil.

Plant associations indicate the different subtypes of the solonetz soil as follows.

I. Achilleo-Festucetum pseudovinae (Magyar) Soó	Deep layer meadow solonetz
II. Artemisio-Festucetum pseudovinae Soó	Medium meadow solonetz
III. Artemisio-Festucetum pseudovinae Soó	Meadow solonetz with degraded A-layer
IV. Camphorosmetum annuae (Rapaics) Soó	Crusted meadow solonetz
V. Pholiuro-Plantaginetum tenuiflorae (Rapaics) Soó	Crusted meadow solonetz
VI. Puccinellietum limosae (Klika) Wendelbg.	Crusted solonetz with precipitated silicate
VII. Agrosti-Beckmannietum eruciformis (Rapaics) Soó	Meadow like solonetz with humic upper soil layer

Collection and processing of samples

Samples were taken in the sites described above with sterile tools from 1.5×1.5 m quadrats. Part-samples were taken from three points of each quadrat, from depths of 0-5, 5-10 and 10-20 cm-s. After a careful mixing of the part-samples taken from the same depth, they (altogether about 1 kg each) were carried to the laboratory in sterile boxes. The homogenised samples were dried on room-temperature for 8-10 days.

During the quantitative analysis of soil samples the culture-dilution method was used. Our method is based on the dilution technique of Varga and Telegdy-Kovács (1953), and also that of Singh (1955) with certain modifications, but incorporates several elements of modifications introduced by Brunberg-Nielsen (1968) and Buitkamp (1979). During our studies this method was used consistently. In our opinion this method can be successfully used in biological analysis of other (not alkaline) soil types.

In the course of the quantitative analysis of soil samples, the culture method has been modified, by which the starting mass of the dry soil used was increased to 5×10 g (Buitkamp, 1979; Foissner, 1981, used 8×3 g soil to start). To wet the soil samples 1:5, Protozoon-free soil extractum was used, by which the drastic destroying influence of distilled water can be eliminated. Levels of dilution ($1/5$ - $1/640$) and repetitions within the levels (10) were optimised.

During the analysis the volume of the samples processed has been increased from 0.05 ml to 1 ml. These modifications have significantly increased the objectivity of the statistical method used, and errors deriving from the aggregated appearance of cysts could be avoided (Gellért, 1957; Stout, 1962). 5×80 cultures were set up from each sample. Culture tubes were incubated on 21°C for 6 days (Buitkamp, 1979). Processing of data was based

on the VIII/2. Table of Fisher-Yates (1963) and on the formula (ind/g) of Brunberg-Nielsen (1968).

After heat treatment – on 58 °C for 45 minutes – the number of cysts has been determined from the soil samples, making the determination of the actual number of species and individuals possible.

For the qualitative analysis of the samples, cultures were set up in every case (Foissner, 1981, 1987). 50 g of the air-dry soil sample was put into a crystallizing cup in a step-like pattern. To wet the soil samples Protozoon-free, 1:5 soil extractum was used. Samples were examined in 2,4,6,10,14 and 21 days, when the appearing species were recorded.

In order to support the identification of species, the wet silvering method of Klein (1926), the core staining method of Feulgen, the silvering method of Chatton-Lwoff (1936) and the protargol method modified by Wilbert (1975) were used.

The estimated biomass of Ciliata in the soils was calculated by the formula of Pussard (1967, 1971) and given in g/ha. The average mass of solonetz soils was considered to be 1.45 g/cm³. Volume of Ciliata has been calculated by the Simpson-formula (Czorik, 1968) and using the method of Buitkamp (1979, and also pers. comm.). The body of the organisms has been compared to simple geometric bodies. The density of the protoplasm has been considered to be 1.

The Simpson-formula is as follows:

$$V = h/6 \times (b_1 + 4b_2 + b_3)$$

(where: V = body volume, h = body length, b₁ = area of the lower segment, b₂ = area or diameter of the middle segment, b₃ = area of the upper segment.)

The estimated biomass of Ciliata in the soils to an area unit (ha) was calculated by the formula of Pussard (1967, 1971):

$$S \text{ (g/ha)} = V \times N \times \delta \times c \times 10^8$$

(where: S = estimated biomass of a species (g/ha) V = volume of the species in 10⁻⁸ cm³, δ = volume weight of the soil (g/cm³), c = thickness of the soil layer examined (cm), δ x c 10⁸ = weight of 1 ha soil (g).)

The average mass of solonetz soils was considered to be 1.45 g/cm³. Results are given in g/ha.

Results and discussion

Based on our studies, there are diverse Ciliata communities in the solonetz soils of the HNP, where altogether 35 species have been found. Most

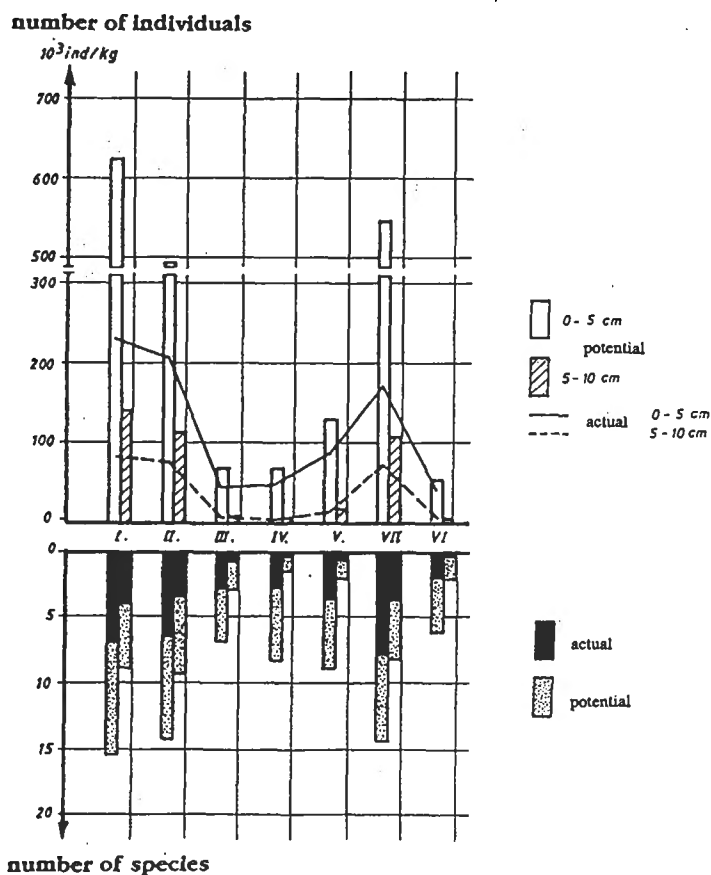


Fig. 1. Number of species and individuals in the sampling sites. (I = deep layer meadow solonetz; II = medium meadow solonetz; III = degraded A-layer crusty solonetz; IV = crusty solonetz; V = crusty solonetz; VI = crusty solodized solonetz; VII = meadow solonetz with humus upper layer)

of the species are well-known, cosmopolitan organisms with wide ecological tolerance spectrum. A lot of species are known from wetlands as well which supports findings of Varga (1936, 1953) based on the ecological classification of whom the Protozoon fauna of the soils is of a limnic origin (hydrobiont edaphon).

In the soils of the HNP the highest numbers of individuals were found in the upper (0-5 cm) layer of the soils of high humous-content (I, II, VI), where it is around 500-600 10^3 ind/kg. This value is only 50% of those coming from the same layer of chernozem soils. In sampling sites with eroded upper layers, abundance of species is rather low (40-60 10^3 ind/kg) in each of the layers studied (Fig. 1).

It was found that in alkaline (solonetz) soils the volume decrease (troglodytism) of Ciliata is significant. Decrease of big organisms reached 30-70%, while that of smaller 20-25%. Calculations on the biomass of Ciliata in the area of the HNP had to be done in each biotope, hence the different subtypes of solonetz soils formulate a mosaic-like pattern.

Because of the difficulties in determining the number of individuals and the mass of the certain species, it was not our aim to calculate the absolute value of the Ciliata biomass, only estimations can be given.

The values of the estimated biomass (kg/ha) show that there is more living material in the upper soil-layers (0-5 cm) than in the lower ones. The estimated biomass is particularly high in those sampling sites (I, II, VII) with a humous-rich upper layer which are more favourable for Ciliata populations than the other (III, IV, V, VI), eroded soil types.

In the soil types with a humous-rich upper layer (A-horizon), covered by *Achilleo-Festucetum pseudovinae*, *Artemisio-Festucetum pseudovinae* and *Agrosti-Beckmannietum eruciformis* plant associations, the estimated biomass is 11-16 kg/ha, based on the cultured population sizes.

In the lower layers of these soils, the decrease in the mass of living material can reach 30-40%.

This significant decrease derives from the unfavourable physical conditions of soils (lack of air, high colloid content, increase of salt concentration, lack of food and water).

In other subtypes and varieties of solonetz soils (crusted, with precipitated silicate) the biomass values are low. Here in absence of a humous-rich A-horizon, the B-horizon comes up to the soil surface and conditions for Ciliata become less favourable, thus the number of species and population sizes are found to be low. The estimated biomass is 1-3 kg/ha in the 0-5 cm layer. In the 5-10 cm layers biomass represented by Ciliata is on the minimum (1.4-0.03 kg/ha).

These data show that there is a positive correlation between the population size of Ciliata and their biomass, which is well-proved by Fig. 1. Because of the small volume of the occurring species, in some sampling sites (III, IV, VI), however, this correlation is not linear!

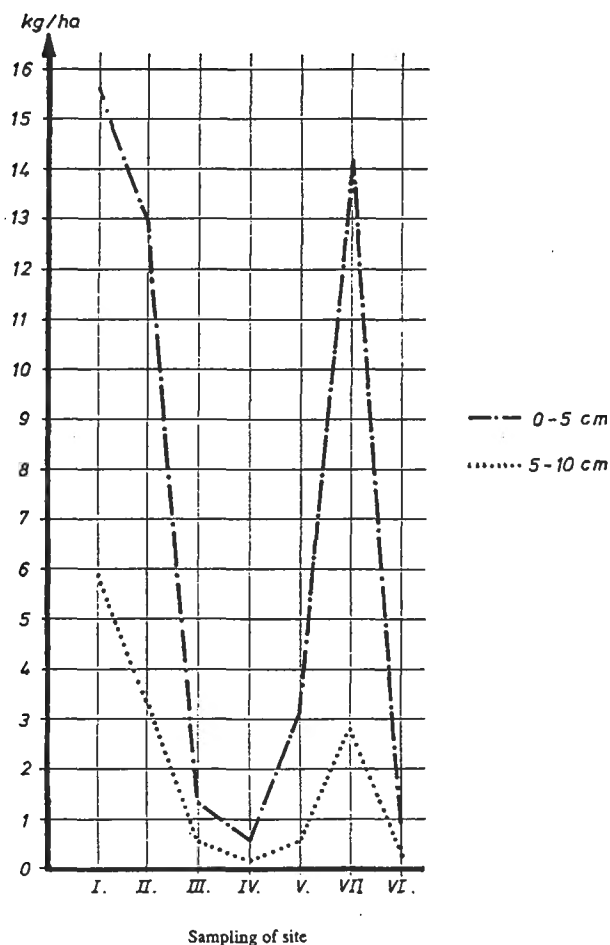


Fig. 2. Changes of the estimated biomass in the different subtypes of solonetz soils

Based on these data, the studied soils can be ordered in a similar way as done by the numbers of species and individuals. Values of the estimated biomass are the highest (Table 1) in the upper (0-5 cm) layer of the deep and medium meadow solonetz soils (I, VII, II).

Table 1. Main characteristics of the Ciliata fauna living in the soils of the Hortobágy National Park with the indication of the estimated biomass (based on average data)

Sampling sites	I.		II.		III.		IV.		V.		VI.		VII.	
Main characteristics	0-5	5-10	0-5	5-10	0-5	5-10	0-5	5-10	0-5	5-10	0-5	5-10	0-5	5-10
Cult. number of indiv. (T) 10 ³ /kg	663	251	455	139	58	14	61	4	117,8	11	554	203	43	10
Cultiv. number of species	15	8	14	9	7	3	8	1	9	2	14	9	6	2
Actual nr. of ind. (T-C) 10 ³ /kg	425,0	169,4	257,0	76,5	25,0	8,4	26,0	1,2	60,8	3,3	381,5	152,2	16	4,3
Actual number of species	7	4	7	3	2,5	0,5	2,6	0,08	3,5	0,7	7,8	3,4	1,8	0,3
Number of cysts (C) 10 ³ /kg	238	1,6	198	62,5	33	5,6	35	2,8	57	7,7	172	50,8	27	5,7
Cyst %	36	33	44	45	57	40	57	70	48	70	31	25	63	57
Hypotrichida %	52	68	53	42	60	100	65	0	64	0	47	41	57	33
Other taxonomic category %	48	32	47	57	40	0	35	85	36	100	53	59	43	66
Biomass kg/ha	16,04	6,84	11,29	3,27	1,24	0,01	0,65	0,03	2,88	0,32	13,6	3,03	0,7	0,0

There is significantly less biomass in the 5-10 cm layer, and in both (0-5, 5-10 cm) layers of the crusted solonetz soils with eroded upper horizon (III, IV, V, VI).

Comparing the estimated biomass values of Ciliata in the soils of the Hortobágy National Park with that of Table 2, it turns out that other authors have only studied the uppermost (0-5 cm) layer of soils. Biomass values given in these publication show an extremely significant deviation. Analysing data of Table 2, it is believed that the extremely high values given by Bick (1972) and Foissner (1981) can be accepted only with reservations.

It comes also from the comparison that in the well-structured chernozem soils the number of individuals, and the closely related biomass values are higher in the 5-10 cm layer than in the upper, 0-5 cm one. This can be explained by the more intensive exposition of the upper layer to the physical effects (radiation, heat, wind) and also by the more favourable water-supply of the lower layers.

In the soil samples studied the cultured number of Ciliata is higher by 30-50% than the actual population sizes of soils. Taking into account this data, the estimated biomass in the alkaline soils with humous-rich upper layer is only 5-8 kg/ha in the upper (0-5 cm) soil-horizon. This value decreases to 0.05-0.3 kg/ha in the 5-10 cm layer.

Because of the mosaic pattern of the studied solonetz soils of the Hortobágy National Park, the estimated average Ciliata biomass calculated on the basis of actual numbers of individuals is 6.6 kg/ha in the upper layers. This value is relatively low, but cannot be neglected while studying food chains and flow of nutrients.

Table 2. Amount and biomass of Protozoa in different soil types

Author's name	Name of the soil	Amoeba	Protozoa n 10 ³ Flagell.	Ind/kg Ciliata	Protozoa n 10 ³ ind/kg	Biomass g/ha	Depth cm
CUTLER (1920)	Arable land	10-60.000	1.000- 10.000	1.000	1.100- 170.000		
CUTLER (1920)	Pasture				5-35.000		
ALLISON	Fertilized soil	1.492	10.314	77-110	11.883		
PERÉY (1925)	Garden soil	446.400	1.030.000	200	1.476.600		
SANDON (1928)	Fertilized soil			15-186	480.000		
BRODSKY (1929)	Desert clay				10-3.000		
TELEGDY-K. (1934)	Arable land	500.000	250.000	5.000	305.000		
DIXON (1936)	Steppe	121.938	443.159	86	565.183		
RUSSEL (1936)	Fertilized soil	150.000	350.000	100	500.100		
HEINIS (1937)		280.000	770.000	1.000	1.071.000		
HEINIS (1937)				26			
SINGH (1946)	Soil from Rothamsted	41.400	70.500	377	112.277		
BREZINKA-D. (1954)	Rice soil	64.500					
BREZINKA-D. (1954)	Very humic soil	1.690.000					
VARGA (1954)	Arable land				150.000		
VARGA (1954)	Forest soil				50.000		
GELLÉRT (1957)	Broad-leaved forest soil			6.450			
GELLÉRT (1957)	Coniferous forest soil			18.500			
RUSSEL (1957)						16.700	
BRUNBERG-N. (1968)	Beech forest soil			300.000			
PRASAD (1968)	Tropical soil				8.873.000		
BICK (1972)	Soil from Matador	20.000	15.000	2.700	37.000	930	0-0,5
		5.500	500	65		2.530	0,5-10
BUTTKAMP (1979)	Pasture			1862		16.682	0-5
	Mixed forest soil			2.029		43.723	0-5
	Tropical sav. forest soil			2.935		16.716	0-5
	Tropical savanna			844		9.964	0-5
	Gallery forest			1.191		9.763	
FOISSNER (1981)	High mountain soil			8.839		66.950	0-5
SZABÓ A. (1991)	Deep layer solonetz			1.549		5200	0-5
	Solonetz			663		16.040	0-5
				251		6.840	5-10
	Crusted meadow solonetz			43		0.700	0-5
				10		0.030	5-10
	Chernozem soil			1.323		24.380	0-5
				2.206		42.400	5-10

It is not possible to compare our data with those of other authors, hence there are no similar results, based on the analysis of alkaline soils neither among Hungarian nor in foreign references.

By the comparison, however, it is remarkable that the estimated biomass of alkaline soils is far behind the values measured in other soil types.

Acknowledgments. Special thanks are due to Mr. Gábor Szilágyi for translating and editing the manuscript. This work was supported by the projekt „OTKA: T 029359”.

REFERENCES

1. BICK, H. (1972): Soil Protozoa. - 5th Annual Report of the Matador Project, Saskatoon: 51-54.
2. BRUNBERG-NIELSEN, L. (1968): Investigations on the microfauna of leaf litter in a Danish beech forest. - *Natura Jutlandica*, 14: 79-89.
3. BUITKAMP, U. (1979): Vergleichende Untersuchungen zur Temperaturadaptation von Bodenciliaten aus klimatisch verschiedenen Regionen. - *Pedobiologia*, 19: 221-136.
4. CHATTON, E. & LWOFF, A. (1936): Impregnation par diffusion argentine de l'infiltration des ciliés marins et d'eau douce, après fixation cytologique et sand dessication. - *C. R. Soc. Biol.*, 104: 834-836.
5. CZORIK, F. P. (1968): Swobodnoskiwuschtschie infusorii wodoemow Moldavii. - *Kishinov*, 36.
6. FISHER, R. A. & YATES, F. (1963): Statistical tables for biological, agricultural and medical research. - Oliver-Boyd, 6th ed., Tabl. VIII, 2.
7. FOISSNER, W. (1981): Die Gemeinschaftsstruktur der Ciliatenzönose in alpinen Böden (Hohe Tauern, Österreich) und Grundlagen für eine Synökologie der terricolen Ciliaten (Protozoa, Ciliophora). - Veröff. Österr. Mass. Hochgebirgsprogramms Hohe Tauern, 4: 7-52.
8. FOISSNER, W. (1998): An update compilation of world soil Ciliates (Protozoa, Ciliophora), with ecological notes, new records, and descriptions of new species. - *Europ. J. Protistol.*, 34: 195-235.
9. FOISSNER, W., BERGER, H., BLATTERER, H. & LOHMANN, F. (1995): Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band IV: Gymnostomatea, Loxodes, Suctorina. - Informationsber. des Bayer. Landesamt für Wasserwirtschaft, 1/95, 540 pp.
10. GELLÉRT, J. (1957): Néhány lomblevelű és tűlevelű erdő talajának Ciliata faunája. - *Annal. Biol. Tihany*, 24: 11-34.
11. PUSSARD, M. (1967): Les protozoaires du sol. Systématique, technique d'étude et de numération, importance et fluctuations numériques, caractères adaptifs. - *Ann. Epiphyties*, 18: 335-360.
12. PUSSARD, M. (1971): Les protozoaires du sol. - In: Pesson, P.: *La vie dans les sols*. Paris: 149-185.
13. SEBESTYÉN, O. (1958): Mennyiségi planktontanulmányok a Balatonon. VII. Biomassza számítások nyíltvízi Oligotricha Ciliátákon - *Annal. Biol. Tihany*, 25: 257-266.
14. SINGH, B. N. (1955): Culturing soil Protozoa and estimating their numbers in soil. - *Soil Zoology*: In: Kevan. London: 403-411.
15. STOUT, J. D. (1962): An estimation of microfaunal populations in soils and forest litter. - *J. Soil. Sci.*, 13: 314-320.
16. VARGA, L. (1960): Über die Mikrofauna der Waldstreu einiger auf Szikböden angelegter Waldtypen. - *Acta Zool. Acad. Sci. Hung.*, 6: 211-225.
17. VARGA, L. & TELEGDY-KOVÁTS, L. (1953): A talajlakó állatok kimutatására és számolására alkalmas módszerek. - In: Ballenegger R. (ed.): *Talajvizsgáló módszer-könyv*. Budapest: 353-374.
18. WILBERT, N. (1975): Eine verbesserte Technik der Protargolimprägnation für Ciliaten. - *Mikrokosmos*, 64: 171-179.